2' OR 3'-DEOXY AND 2', 3'-DIDEOXY-β-L-PENTOFURANONUCLEOSIDE COMPOUNDS, METHOD OF PREPARATION AND APPLICATION IN THERAPY, ESPECIALLY AS ANTI-VIRAL AGENTS

The subject of the present invention is a method for the stereospecific preparation of 2'- or 3'-deoxy- and 2',3'-dideoxy-β-L-pentofuranonucleoside compounds.

The present invention also relates to 2'- or 3'-deoxy- and 2',3'-dideoxy-β-L-pentofuranonucleoside compounds.

The present invention finally relates to the use of these compounds as drugs and especially as antiviral agents.

Up until now, the synthesis and the biological evaluation of nucleoside analogs of the L configuration have been the subject of some studies, but until recently, the activities of most of the nucleosides were only associated with those of their D isomers [A. Holy, in Synthesis, Structure and Chemistry of Transfer Ribonucléic Acids and their Components (Proceedings of the International Conference Held in Dymaczewo near Poznan, Poland on September 13-17, 1976), Polish Academy of Sciences, Poznan, 1976, p. 134, and references cited]. However, recently, β-L-Thymidine [S. Spadari, G. Mage, F. Focher, G. Ciarrocchi, R. Manserwigi, F. Arcamone, M. Capobianco, A. Carcuro, F. Colonna, S. Iotti and A. Garbesi, J. Med. Chem. 35, 4214 (1992)] and 2',3'-dideoxy-β-L-cytidine (β-L-DDC) [M.M. Mansuri, V. Farina, J.E. Starret Jr., D.A. Benigni, V. Brankovan and J.C. Martin, Bioorg. Med. Chem. Letters, 1, 65 (1991)] have been shown to exert a relatively limited antiviral activity in cell cultures against herpes simplex viruses (HSV) and the human immunodeficiency virus (HIV), respectively, the latter being the causative agent of the acquired immunodeficiency syndrome (AIDS).

In reality, β-L-DDC has been previously reported in a contradictory manner, on the one hand, to have no activity against HIV [M. Okabe, R.C. Sun, S.Y.-K. Tam, L.J. Todaro and D.L. Coffen, J. Org. Chem. 53, 4780 (1988)] and, on the other hand, to still exhibit a

moderate activity against HIV [IC_{50} = 0.66 10^{-6}M in a CEM cell culture: M.M. Mansuri, Y. Farina, J.E. Starret Jr., D.A. Benigni, V. Brankovan and J.C. Martin, Bioorg. Med. Chem. Letters, 1, 65 (1991)].

On the other hand, analogs of L isomers of AZT have been tested and have appeared to be inactive as anti-HIV agent [J. Org. Chem. 56, 3591 (1991)].

Accordingly, in a β-L isomeric series, nucleotide analogs of the dioxolanyl type [H.O. Kim, R.F. Schinazi, K. Shanmuganathan, L.S. Jeong, J.W. Beach, S. Nampalli, D.L. Cannon and C.K. Chu, J. Med. Chem. 36, 519 (1993) and references cited] and of the oxathiolanyl type [L.S. Jeong, R.F. Schinazi, J.W. Beach, H.O. KIM, S. Mampalli, K. Shanmuganathan, A.J. Alues, A. McMillan, C.K. Chu and R. Mathis, J. Med. Chem. 36, 181 (1993), and references cited] have been proposed and have been found to exhibit an anti-HIV activity.

The present invention provides new compounds which are nucleoside analogs with β anomers and which are of L configuration. Among these L enantiomers, a small number of examples of β-L-2',3'-dideoxynucleosides have been reported in the literature, but are based on methods of synthesis always involving a separation of the α anomers. [Patent EP 352 248 A1 24 Jan. 1990 (CA: 113(5), 41231 w (1990)]; Patent EP 285 884 A2 12 Oct. 1988 (CA: 111 (3), 23911x (1989)] and/or of their D enantiomers. [Patent JP 02 069 469 A2, 8 March 1990 (CA: 115(1), 8560w (1991)]; Patent JP 0 222 9192 A2, 11 September 1990 [CA: 114(11), 102709c (1991)]; Patent JP 0206 9476 A2 8 March 1990 [CA: 113(11), 97977m (1990)]; L. Kaulina, E. Liepins, M. Lidaks and R.A. Zhuk, Khim. Geterstsikl - Soedin. (1), 101 (1982) [CA: 96(17), 143 248e (1982)], which are obtained concomitantly, such that their stereospecificity or their isomeric purity can be questioned.

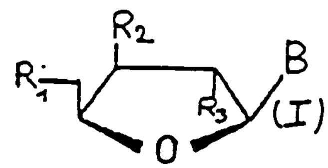
In particular, Patent EP 352 248 describes a method of synthesis by condensation of a sugar and a purine or pyrimidine base.

However, in Patent EP 352 248, the initial sugar compound has a hydrogen and a halogen at the 2' position,

such that the condensation with the base B leads to a mixture of α and β anomers.

The present invention provides a method which makes it possible to prepare stereospecifically the β-L-2',3'-dideoxynucleoside compounds. It appeared, after evaluation of their potential as antiviral agent, more particularly against HIV, that some of these stereoisomeric compounds were particularly active.

The subject of the present invention is therefore firstly a method for the preparation of 2'- or 3'-deoxy- and 2',3'-dideoxy-β-L-pentofuranocucleoside compounds of formula I:

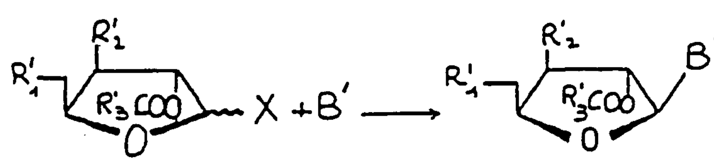


in which

- B represents a purine or pyrimidine base;
- R_1 represents OH;
- R_2 and R_3 represent, independently of each other, H or OH;
- at least one of R_2 and R_3 represents H;

characterized in that the following steps are carried out:

1) a compound of formula (II) is condensed with the base B in order to obtain the compound of formula (III)

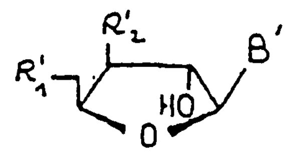
(II)  (III)

in which formulae (II) and (III)

- R'_1 and R'_2 have the meanings given for R_1 and R_2 except that when R_1 and R_2 represent OH, the said OH group is protected by a protecting group such as an acyl, benzoyl, benzyl or silyl group,

- R'_3 represents a C_1 to C_5 alkyl group or a phenyl radical, which are optionally substituted,
- X is a leaving group such as Cl, Br, I or a C_1 to C_5 acyloxy or alkoxy group,
- B' is a purine or pyrimidine base B which is optionally appropriately protected,

2) the R'_3 CO group at the 2' position is removed by deacetylation so as to obtain an OH group and a compound of formula



(IV)

3) optionally, the OH group at the 2' position is removed;

4) where appropriate, the R'_1 and R'_2 groups and the B' base are deprotected so as to obtain the compounds of formula (I).

The presence of an acyl protection at the 2' position causes a stereospecific coupling with the heterocyclic base leading stereospecifically to the β anomer of the nucleoside during the glycosylation according to the Baker "trans" rule, because it induces the formation of an intermediate acyloxonium.

Any heterocyclic base can be condensed with the sugar (II). There may be mentioned in particular B represents one of the adenine, guanine, hypoxanthine, uracil, thymine or cytosine bases, it being possible for these bases to be substituted especially by halogen at the 5 position for uracil and cytosine.

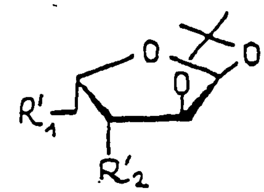
For each type of base, the conditions for glycoside condensation in nucleoside synthesis are numerous and well known to persons skilled in the art.

Instead of the above step 3) for removal, a substitution of the OH group by an N_3, F or NH_2 group can be carried out with, in this case, an inversion of configuration on the carbon considered. Compounds of formula (I) are obtained in this cas with $R_2 = N_3$, F or

NH_2 with the inverse configuration to that represented in formula (I).

Preferably, in the compounds (II) and (III), R'_3 represents a C_1 to C_5 alkyl group, preferably CH_3.

Thus, the compounds (II) can be prepared in which the compound (II), di-O-acetylated at the 1, 2 position, in which X and R'_3COO represent an O-acetyl group, is prepared by acetolysis of the 1,2-isopropylidene-L-xylofuranose compound of formula (V)

(V) 

This reaction occurs in two stages:

a) in an acidic medium CH_3COOH 85% and H_2SO_4, then

b) with $(CH_3CO)_2O$ in pyridine.

Preferably, R'_2 and R'_3COO are different, in particular R'_2 is an O-benzoyl group and R'_3COO is an O-acyl group.

Thus, it is possible to selectively deprotect the alcohol at the 2' position by means of hydrazine hydrate at step 2) above.

Scheme I outlines the various steps of the synthesis of compounds of formula (I) in which R_2 and R_3 represent H or OH by detailing the reaction conditions of persons skilled in the art. It is possible to introduce the compounds of formula (I) onto the N_3, F and NH_2 groups by substitution in place of the OH group with inversion of configuration.

In Scheme I, starting with commercial L-xylose, two synthesis routes are described, both involving the prior production of an L-pentofuranose (compounds <u>3</u> and <u>14</u>) appropriately protected and possessing at the 2 position a participating O-acyl group inducing during glycosylation reactions the 1',2'-Trans geometry desired for the nucleoside obtained (B.R. Baker, in the Ciba Foundation Symposium on the Chemistry and Biology of th

Purines, G.E.W. Wrolstentiolme and C.M. O'Connor Eds, Churchill London, p. 120 (1957)). The sugar 3 and 14 are obtained from L-xylose which is converted to 1,2-isopropylidene-L-xyloruranose, the acetolysis of which leads to the di-O-acetylated derivative at the 1,2 position.

Compound 1 is obtained in two stages from acetone in the presence of copper sulfate and then in acid medium.

Compound 1 is reacted with C_6H_5COCl in pyridine in order to obtain the one intermediate whose OH groups at the 5′ and 3′ positions are protected by a benzoyl. This protected intermediate is acetolyzed in acid medium (CH_3COOH 85%, H_2SO_4) and then in the presence of anhydride $(CH_3CO)_2O$ in pyridine in order to give the compound 3.

The first synthesis route consists in condensing 1,2-di-O-acetyl-3, 5-di-O-benzoyl-L-xylofuranose (3) with a heterocyclic base. The nature of the acetyl protecting group of the sugar 3 at the 2′ position causes a stereospecific coupling leading to the β anomer. The protecting group at the 3′ position being different, it is possible to then deacetylate selectively at the 2′ position by means of hydrazine hydrate in pyridine in acid medium the completely protected β-L-xylofuranosyl-nucleoside 4 obtained, thus leading to the compound 5. The latter is then converted to its thiocarbonyl derivative which is subjected to a Barton-type deoxygenation reaction involving free radicals according to an experimental method which is already used in the D series (M.J. Robins, D. Nadej, F. Hansske, J.S. Wilson, G. Gosselin, M.C. Bergogne, J.L. Imbach, J. Balzarini and F. De Clercq, Can. J. Chem. 66, 1258 (1988)), in order to give the compound 6. The Barton reduction involving free radicals consists in substituting the hydrogen of the alcohol functional group by a C(S)X group (X = imidazole, phenoxy and the like) and then in reducing the ROC(S)X functional group by homolytic rupture by means of Bu_3SnH and AIBN. Compound 6 is debenzoylated in order to give the 2-deoxy-β-L-threopentofuranosylnucleoside 7. A sel ctive protection of the 5′ primary hydroxyl leads to

the derivative 8 which is subjected to a deoxygenation at the 3' position according to the Barton-type method. Finally, th resulting compound 9 is deprotected at the 5' position in order to give the 2',3'-β-L-pentofuranosylnucleoside 10.

The second synthesis route involves the prior preparation of 1,2-di-O-acetyl-3-deoxy-5-O-benzoyl-L-erythropentofuranose (14), unpublished up until now. To do this, the 1,2-di-O-isopropylidene-α-L-xylofuranose (1), obtained in two stages from L-xylose and which is an intermediate in the synthesis of the sugar 3 used in the first route, is selectively benzoylated at the 5 position in order to give the compound 11. The latter is converted to its thiocarbonylated derivative 12 which is deoxygenated by means of trimethylsilylsilane (D.H.R. Barton, D.O. Jang and J. Cs. Jaszberenyi, Tetrahedron, 49, 2793 (1993)) in order to give the compound 13. This compound 13 is freed of acetone in aqueous acetic acid in the presence of sulfuric acid, and the resulting intermediate is not isolated, but directly acetylated using acetic anhydride in pyridine in order to give the desired sugar 14. The condensation of 14 with a purine or pyrimidine aglycone leads to the protected nucleoside 15 which can be either completely deacylated by means of sodium methoxide or ammonia in solution in methanol to give the 3-deoxy-β-L-erythropentofuanosylnucleoside 16, or be selectively deacetylated at the 2' position by means of sodium methoxide in THF in order to give the derivative 17. A deoxygenation reaction according to Barton on the 2'-thiocarbonylated derivative of 17 then leads to the derivative 9, which is identical to that obtained in the first synthesis route.

To illustrate the present invention, the preparation (according to the first synthesis route) and the characterization of 2'-3'-dideoxy-β-L-uridine (β-L-DDU), 10a) and that of 2',3'-dideoxy-5-fluoro-β-L-uridine (β-L-5-fluoro-DDU), 10b) are described in the examples which will be given below.

To prepare a compound of formula (I) in which B

is cytosine, it is possible, according to the present invention, to prepare a compound of formula (I) where B is uracil, and then to convert the uridine derivative to a cytidine derivative by converting the uracil to cytosine.

The experimental conditions are indicated in Scheme II which represents the conversion of β-L-DDU and β-L-5-fluoro-DDU. These two compounds are converted (Scheme II) to 2',3'-dideoxy-β-L-cytidine (β-L-DDC, 21a) and to 2',3'-dideoxy-β-L-5-fluorocytidine (β-L-5-fluoro-DDC, 21b), respectively.

The β-L-DDU (10a) and β-L-5-FDDU (10b) are selectively acetylated at the 5' position in order to give the compounds 18a, b. The latter are converted to their corresponding thioamide derivatives 19a, b by treatment with the Lawesson reagent at reflux in dichloroethane according to a method previously developed in the D series of uridine (J.E. Starrett, Jr., D.R. Tortolani, D.C. Baker, M.T. Omar, A.K. Hebbler, J.A. Wos, J.C. Martin and M.M. Mansuri, Nucleosides Nucleotides, 9, 885 (1990)). The compounds 19a,b are treated with ammonia in methanol either at room temperature, or at 100°C in order to give, respectively, the desired 2',3'-dideoxy-β-L-4-thiouridine 20a and its 5-fluorinated derivative 20b, as well as β-L-DDC (21a) and β-L-5-FDDC (21b).

While the L isomers are considered to be less toxic than the D isomers, because apparently they do not cause the same mutations in reverse transcriptase, the negative results obtained in the prior art, as regards the antiviral activity of the compounds of the 2',3'-L-dideoxynucleoside type (absence of activity or low activity) could be linked to a defect in stereospecificity.

Indeed, the β-L-DCC (21a) and β-L-5-F-DDC (21b) which are obtained according to the present invention have been evaluated against HIV in cell cultures (see examples below) on which these two molecules proved active, with especially a very high antiviral activity

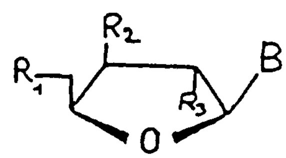
for β-L-5-FDDC.

In addition, 2',3'-dideoxy-β-L-5-fluorocytidine appears to be active on strains which are resistant to AZT and to nevirapine, the latter being at present the subject of clinical trials.

The compounds, in which one of R_2 or R_3 is OH and the other is H, can constitute intermediate compounds which are useful in the synthesis of the 2',3'-dideoxy-β-L-nucleoside derivatives, variously substituted on the sugar, especially by N_3, F or NH_2 groups.

In particular, the 2'-deoxy compounds of "threo" configuration (see in particular Scheme I, the compounds 7) and the 3'-deoxy compounds of "erythro" configuration (see in particular Scheme I, the compounds 16 upon deoxygenation of their hydroxyl lead to the β-L-2',3'-dideoxynucleosides.

The subject of the present invention is also stereochemically pure stereoisomeric β-L-pentofuranonucleoside compounds corresponding to the following formula



(I)

in which

- R_1 and B have the meanings given above, and either R_2 represents OH and R_3 represents H, or R_2 represents H and R_3 represents OH.

Indeed, the β-L-pentofuranonucleoside compounds described in the literature always comprise an inverse configuration 3' ribo instead of 3' xylo as in the present invention. There is no example which is described in the literature of compounds of formula (I) with R_2 = H and R_3 = OH.

There may be mentioned in particular the compounds for which B represents uracil, 5-fluorouracil, hypoxanthine, 5-fluorocytosine guanine or adenine.

Th subject of the present invention is also

2',3'-dideoxy-β-L-pentofuranonucleoside compounds of formula (I) above, in which

. R_1 represents OH

. R_2 and R_3 represent H and

. B represents uracil, guanine, hypoxanthine, 5-fluorouracil, 5-fluorocytosine.

The subject of the present invention is more particularly a compound chosen from β-L-ddU, β-L-5-fluoro-ddU, β-L-5-fluoro ddC.

Finally, the subject of the present invention is the use of the compounds according to the invention as drugs. These may be, depending on the cases, antibiotics, antitumor agents or antiviral, especially anti-HIV, agents. In particular, as regards the 2',3'-dideoxy-nucleoside compounds according to the invention, these are more particularly useful as antiviral agent.

The subject of the present invention is especially the therapeutic application in antiretroviral chemotherapy of β-L-5-F-DDC, more particularly as anti-HIV agent.

Further advantages and characteristics of the present invention will appear in the light of the examples which will be given below.

Figure 1 represents Scheme I.

Figure 2 represents Scheme II.

<u>EXAMPLE 1:</u> <u>Preparation of 1,2-di-O-acetyl-3,5-di-O-benzoyl-L-xylofuranose (3)</u>

The procedures and the materials used have been described in J. Chem. Soc., Perkin Trans. I 1943 (1992).

The L-xylose was bought from Interchim, France.

This compound 3 is prepared in four stages from L-xylose without purification of the intermediates.

According to the same experimental procedure as that described in Gosselin et al., Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques, PT4, L.B. Towsend and R.S. Tipan, eds. John Witey and Sons, Inc., 1991, p. 41.

The monomeric mixture of 3 was obtained in the form of a pale y llow syrup and a recrystallization from

ethanol led to the pure α anomer (yield 26%) melting point 104-107°C.

NMR data (DMSO, d): δ 2.06 and 2.10 (2 s, H.3 each, 2 $COCH_3$), 4.50 (m, H2, H5, 5'), 4.85 (m, 1H, H.1), 5.54 (dd, 1H, H-2, J = 4.6 and 5.79 Hz (t, 1H, H.3, J = 6.3 Hz)), (6.43 d, 1H, H.1, J = 4.6 Hz), 7.5 - 8.0 (m, 10H, 2 COC6H5); $[\alpha]_D^{20}$ -125.2°(C) 1,3 $CHCl_3$]; mass spectrum: (FAB > 0 3-nitrobenzyl alcohol matrix) m/z 443 [M + HJ$^+$, 383 [383 [M - CH_3CO_2], 105 [C_6H_5C = 0]

Calc. for $C_{22}H_{22}O_9$ (442.41): C: 62.44; H: 5.01 - Found: C: 62.28 and H: 5.04.

EXAMPLE 2: Preparation of 1-(2-0-acetyl-3,5-di-O-benzoyl-β-L-xylofuranosyl)uracil (4)

Hexamethyldisilazane (1.9 ml; 9.01 mmol), trimethylchlorosilane (1.15 ml; 9.06 mmol) and tin (IV) chloride (1.59 ml; 13.5 g mmol) were added successively to a mixture of uracil (1.27 g; 11.33 mmol) and protected sugar (3) (5.0 g; 11.30 mmol), in anhydrous acetonitrile (170 ml). The clear solution obtained was stirred at room temperature for 24 hours. The reaction mixture was concentrated to a reduced volume, then diluted with chloroform (150 ml) then washed twice with the same volume of a solution of aqueous sodium hydrogenate carbonate and finally with water. The organic layers were dried over sodium sulfate, filtered on celite and then evaporated. The product obtained was purified on a silica gel chromatography column [eluent: gradient of methanol (0.4%) in methylene chloride] to give the pure compound 4 (3.709, 66%).

GENERAL CONDITIONS AND INSTRUMENTATION USED: They are identical to those reported by C. Périgaud, G. Gosselin and J.-L. Imbach, *J. Chem. Soc., Perkin Trans.* 1, 1943 (1992).

Example 3: 2',3'-Dideoxy-β-L-uridine (β-L-DDU, 10a).

1-(2-Deoxy-β-L-*threo*-pentofuranosyl)uracil (7a). A solution of 1-(3,5-di-O-benzoyl-β-L-xylofuranosyl)-uracil (6a)(1.4 g : 3.21 mmol) in ammoniacal methanol (previously saturated at 10°C and hermetically closed)

(90 ml) is stirred for two days at room temperature. The solution is evaporated several times with methanol under reduced pressure. The crude material obtained is dissolved in water and the resulting solution is washed several times with chloroform. The aqueous phase is evaporated and the residue is directly crystallized from methanol to give 0.6 g (yield 82%) of pure 7a: m.p.: 165-167°C; ^{1}H NMR (DMSO-d_6), δ ppm = 11.23 (s, 1H, 3-NH), 7.92 (d, 1H H-6; J = 8.1 Hz), 6.04 (dd, 1H, H-1'; J = 2.0 and 8.3 Hz), 5.26 (d, H, H-5; J = 8.1 Hz), 5.26 (d, 1H, OH-3'; J = 3.2 Hz), 4.69 (t, 1H, OH-5'; J = 5.3 Hz), 4.20 (m, 1H, H-3'), 3.81 (m, 1H, H-4'), 3.80-3.60 (m, 2H, H-5' and 5"), 2.6-2.5 (m, 1H, H-2', partially obscured by DMSO-d_5), 1.85 (dd, 1H, H-2"; J = 2.0 and 14.7 Hz); mass spectra (matrix: glycerol-thioglycerol: 50:50, v/v): FAB>0 321 $[M+glycerol+H]^+$, 229 $[M+H]^+$, 117 $[s]^+$ and 113 $[BH_2]^+$; FAB<0 227 $[M-H]^-$,

Anal. Calculated for $C_9H_{12}N_2O_5$ (M = 228.21): C 47.36; H 5.30; N 12.28. Found: C 47.45; H 5.46; N 12.12.

1-(5-O-Tert-butyldiphenylsilyl-2-deoxy-β-L-*threo*-pentofuranosyl)uracil (8a).

Tert-butyldiphenylsilane chloride (0.9 ml; 3.50 mmol) is added to a solution of 7a (0.6 g; 2.63 mmol) in anhydrous pyridine (8 ml). The solution is stirred for 4 h at room temperature and then the solvent is evaporated under reduced pressure. Water and dichloromethane are added. The organic phase is separated, washed successively with a saturated aqueous sodium hydrogen carbonate solution and water, dried over sodium sulfate and filtered. After evaporation to dryness, the residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-2%) in dichloromethane] to give 1.2 g (98%) of 8a in the form of a foam: ^{1}H NMR (DMSO-d_6) δ ppm: 11.26 (s, 11H, 3-NH), 7.78 (d, 1H, H-6; J = 8.2 Hz), 7.65-7.35 (m, 10 H, 2 C_6H_5), 6.09 (dd, 1H, H-1'; J = 1.9 and 6.4 Hz), 5.55 (d, 1H, H-5; J = 8.2 Hz), 5.30 (d, 1H, OH-3': J = 3.1 Hz), 4.25 (m, H, H-3'), 4.05-3.95 (m, 2H, H-4' and 5'), 3.85-3.80 (m, 1H, H-5"), 2.6-2.5

(m, 1H, H-2′ partially obscured by DMSO-d_5), 1.85 (dd, 1H, H-2″; J = 1.9 and 16.5 Hz), 0.93 [s, 9H, $(CH_3)_3C$]; mass spectrum (matrix: glycerol-thioglycerol: 50:50, v/v): FAB<0 465 $[M-H]^-$ and 111 $[B]^-$.

5′-O-Tert-butyldiphenylsilyl-2′,3′-dideoxy-β-L-uridine (9a).

O-Phenylchlorothionoformate (0.68 ml; 5.02 mmol) and 4-dimethylaminopyridine (DMAP) (2.64 g; 21.6 mmol) are added to a solution of 8a (1.1 g; 2.36 mmol) in anhydrous acetonitrile (66 ml). The solution is stirred overnight at room temperature and then the solvent is evaporated under reduced pressure. Dichloromethane and water are added. The organic phase is separated and then washed successively with a 0.5M cooled aqueous solution of hydrochloric acid, water and a saturated aqueous solution of sodium hydrogen carbonate and again with water before being dried over sodium sulfate, filtered and evaporated to dryness. The residue (1.9 g) is dissolved in anhydrous dioxane, the resulting solution is evaporated under reduced pressure and this operation is repeated three times in order to give the crude thiocarbonate derivative. The latter is dissolved in dioxane (28 ml) and treated with tributyltin hydride (1.57 ml; 5.83 mmol) and α,α′-azobisisobutyronitrile (AIBN) (0.12 g; 0.73 mmol) at 90°C for 2 h under argon. An additional quantity of Bu_3SnH (0.63 ml; 2.3 mmol) and of AIBN (50 mg; 0.30 mmol) is added and the heating is continued for 30 min. After evaporation of the solvent, dichloromethane and water are added. The organic phase is separated, dried over sodium sulfate and evaporated to dryness. The residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-5%) in dichloromethane] to give 0.54 g (yield 51%) of pure 9a which crystallizes from ethyl ether: m.p. = 145-147°C; UV [[EtOH 95] λmax 264 nm, λmin 235 nm; ^1H NMR (DMSO-d_6) δ ppm: 11.29 (s, 1H, 3-NH), 7.74 (d, 1H, H-6), 7.65-7.40 (m, 10 H, 2 C_6H_5), 5.99 (dd, 1H, H-1′; J = 3.1 and 7.4 Hz), 5.21 (d, 1H, H-5; J = 8.1 Hz), 4.15-4.00 (m, 1H, H-4′), 3.94 (dd, 1H, H-5′; J = 3.0 and 11.3 Hz), 3.75 (dd,

1H, H-5"; J = 4.0 and 11.3 Hz), 2.45-2.20 (m, 1H, H-4'), 2.10-1.85 (m, 3H; H-2", 3' and 3"), 0.99 [s, 9H, $(CH_3)_3C$]; mass spectrum (matrix: glycerol-thioglycerol, 50:50, v/v) FAB>0 451 $[M+H]^+$, 339 $[s]^+$ and 113 $[BH_2]^+$.

2',3'-Dideoxy-β-L-uridin (β-L-ddU: <u>10a</u>).

The compound <u>9a</u> (0.25 g; 0.55 mmol) is dissolved in tetrahydrofuran (1.1 ml) and a 1.1M solution of tetra-*n*-butylammonium fluoride in THF (0.55 ml) is added. The solution is stirred for 2 h at room temperature and then evaporated under reduced pressure. Dichloromethane and water are added, and the aqeuous phase is evaporated to dryness. The residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-5%) in dichloromethane] to give 41 mg (yield 35%) of pure <u>10a</u> which crystallizes from dichloromethane: m.p. = 120-121°C; UV [EtOH 95] max 262 nm, λmin 231 nm; ^1H NMR (DMSO-d_6) δ ppm: 11.24 (s, 1H, 3-NH), 7.93 (d, 1H, H-6; J = 8.1 Hz), 5.93 (dd, 1H, H-1'; J = 3.4 and 6.7 Hz), 5.57 (d, 1H, H-5; J = 8.1 Hz), 5.02 (poorly resolved triplet, 1H, OH-5'), 4.05-3.95 (m, 1H, H-4'), 3.70-3.40 [m, 2H, H-5' and 5"; after exchange D_2O: 3.63 ppm (dd, 1H, H-5'; J = 3.4 and 12.1 Hz) and 3.49 (dd, 1H, H-5"; J = 4.0 and 12.1 Hz), 2.35-2.20 (m, 1H, H-2'), 2.0-1.65 (m, 3H, H-2", 3' and 3"); mass spectra (matrix: glycerol-thioglycerol, 50:50, v/v); FAB>0 425 $[2M+H]^+$, 213 $[M+H]^+$, 113 $[BH_2]^+$ and 101 $[s]^+$; FAB<0 211 $[M-H]^-$ and 111 $[B]^-$.

<u>Example 4</u>: 2',3'-Dideoxy-β-L-5-fluorouridine (β-L-5-FDDU, <u>10b</u>)

1-(2-Deoxy-β-L-*threo*-pentofuranosyl)-5-fluorouracil (<u>7b</u>).

A solution of 1-(3,5-di-O-benzoyl-2-deoxy-β-L-xylofuranosyl)-5-fluorouracil (<u>6b</u>) (0.25 g, 0.55 mmol) in ammoniacal methanol (25 ml) is stirred overnight at room temperature. The solution is evaporated under reduced pressure and the residue is evaporated several times with methanol. The crude material obtained is dissolved in water and the resulting solution is washed several times with chloroform. The aqueous phase is evaporated and the r sidu is dir ctly crystallized from methanol to give

115 mg (yield 85%) of pure <u>7b</u>: m.p. = 198-200°C; UV [EtOH 95] λmax 267 nm (ε, 8500), λmin 233 nm (ε, 2100); ^1H NMR (DMSO-d_6) δ ppm = 11.79 (s, 1H, 3-NH), 8.16 (d, 1H, H-6; J = 7.4 Hz), 6.05 (dd, 1H, H-1'; J = 1.8 and 6.5 Hz), 5.38 (d, 1H, OH-3'; J = 3.3 Hz), 4.73 (t, 1H, OH-5'; J = 5.4 Hz), 4.30-4.20 (m, 1H, H-3'), 3.85-3.60 (m, 3H, H-4',5' and 5"), 2.60-2.50 (m, 1H, H-2' partially obscured by DMSO-d_5), 1.90 (dd, 1H H-2"; J = 1.8 and 14.7 Hz); mass spectra (matrix: glycerol-thioglycerol, 50:50, v/v): FAB>0 339 $[M+glycerol+H]^+$, 247 $[M+H]^+$, 131 $[BH_2]^+$ and 115 $[s]^+$; FAB<0 245 $[M-H]^-$ and 129 $[B]^-$.
Anal. Calculated for $C_9H_{11}N_2O_5F$ (M = 246.20): C 43.90; H 4.51; N 11.38; F 7.72. Found: C 43.60; H 4.57; N 11.22; F 7.40.

1-(5-O-Monomethoxytrityl-2-deoxy-β-L-*threo*-pentofuranosyl)-5-fluorouracil (<u>8b</u>).

4-Methoxytriphenylchloromethane (1.96 g; 6.35 mmol) is added to a solution of <u>7b</u> (1.30 g; 5.28 mmol) in anhydrous pyridine (60 ml). The solution is stirred for 48 h at room temperature and then the solvent is evaporated under reduced pressure. Water and dichloromethane are added. The organic phase is separated, washed successively with a saturated aqueous solution of sodium hydrogen carbonate and water, dried over sodium sulfate and filtered. After evaporation to dryness, the residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-5%) in dichloromethane] to give 2.6 g (yield 95%) of <u>8b</u> in foam form: ^1H NMR (DMSO-d_6) δ ppm: 11.82 (s, 1H, 3-NH), 7.92 (d, 1H, H-6; J = 7.3 Hz), 7.5-6.8 (m, 14H, mMTr), 6.11 (d, 1H, H-1'; J = 7.9 Hz), 5.35 (d, 1H, OH-3'; J = 3.1 Hz), 4.20-4.15 (m, 1H, H-3'), 4.15-4.10 (m, 1H, H-4'), 3.72 (s, 3H, OCH_3), 3.40-3.10 (m, 2H, H-5' and 5"), 2.60-2.45 (m, 1H, H-2' partially obscured by DMSO-d_5), 1.90 (d, 1H, H-2"; J = 14.7 Hz); mass spectrum (matrix: glycerol-thioglycerol, 50:50, (v/v): FAB<0 1553 $[3M-H]^-$, 1035 $[2M-H]^-$, 517 $[M-H]^-$, 245 $[M-monomethoxytrityl]^-$ and 129 $[B]^-$.

5'-O-Monom thoxytrityl-2',3'-dideoxy-β-L-5-fluorouridin (<u>9b</u>).

This compound is prepared according to a method similar to that used during the synthesis of 9a. Thus, 8b (2.8 g; 5.40 mmol) is reacted with O-phenylchlorothionoformate (1.5 ml; 11.08 mmol) and DMAP (5.97 g, 48.85 mmol) in anhydrous acetonitrile (250 ml) to give, after treatment, a residue which is treated with Bu_3SnH (3.75 ml; 13.93 mmol) and AIBN (0.28 g; 1.70 mmol) in dioxane (95 ml) for 2 h under argon. After treatment, the residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-2%) in dichloromethane] to give 1.6 g (yield 59% of 9b in foam form: ^{1}H NMR (DMSO-d_6) δ ppm: 11.84 (s, 1H, 3-NH), 7.90 (d, 1H, H-6; J = 6.8 Hz), 7.50-6.80 (m, 14 H, mMTr), 5.95 (d, 1H, H-1'; J = 5.9 Hz), 4.20-4.10 (m, 1H, H-4'), 3.72 (s, 3H, OCH_3), 3.40-3.15 (m, 2H, H-5' and 5" partially obscured by H_2O), 2.40-2.20 (m, 1H, H-2'), 2.20-1.85 (m, 3H, H-2", 3" and 3"); mass spectrum (matrix: glycerol-thioglycerol, 50:50, v/v); FAB<0 1003 $[2M-H]^-$, 501 $[M-H]^-$, 229 $[M-monomethoxytril]^-$, 129 [B].

2',3'-Dideoxy-β-L-5-fluorouridine (β-L-5-FDDU: 10b).

The compound 9b (1.6 g, 3.18 mmol) is dissolved in 80% aqueous acetic acid and the solution is stirred at room temperature for 2 h. After evaporation of the solvents, the residue is coevaporated several times with a toluene-methanol mixture. A silica gel column chromatography [eluent: step gradient of methanol (0-5%) in dichloromethane] gives 0.6 g (yield 82%) of 10b in foam form: ^{1}H NMR (DMSO-d_6) δ ppm: 11.76 (s, 1H, 3-NH), 8.38 (d, 1H, H-6; J = 7.5 Hz), 5.89 (dd, 1H, H-1'; J = 2.0 and 4.0 Hz), 5.20 (t, 1H, OH-5'; J = 5.0 Hz); 4.10-4.00 (m, 1H, H-4'), 3.80-3.65 (m, 1H, H-5'; after exchange D_2O: 3.69 ppm, dd, J = 2.8 and 12.3 Hz), 3.60-3.50 [m, 1H, H-5"; after exchange D_2O; 3.50 ppm, dd, J = 3.3 and 12.3 Hz], 2.35-2.20 (m, 1H, H-2'), 2.10-1.80 (m, 3H, H-2", 3' and 3"); mass spectra (matrix: glycerol-thioglycerol, 50:50, v/v): FAB>0 231 $[M+H]^+$, 131 $[BH2]^+$, 101 $[s]^+$; FAB<0 229 $[M-H]^-$, $[B]^-$.

Example 5: 2',3'-Dideoxy-b-L-cytidine (β-L-DDC, 21a).

Acetic anhydride (0.27 ml; 2.9 mmol) is added at 0°C to a solution of β-L-DDU (10a) (0.4 g; 1.88 mmol) in anhydrous pyridin (6 ml) and the reaction mixture is stirred for 1 h at 0°C, and then for 5 h at room temperature. Ice-cold water is then added and the mixture is extracted twice with chloroform. The organic phase is dried over sodium sulfate, filtered, coevaporated several times with toluene and evaporated to dryness to give 0.55 g of a residue corresponding to 5'-O-acetyl-β-L-DDU (18a) which is sufficiently pure (tlc) to be used directly in the next stage. Lawesson's reagent (Aldrich, Item 22,743-9; 0.64 g; 1.6 mmol) is added to a solution of the residue in anhydrous dichloroethane (49 ml) and the mixture is heated at reflux under argon for 2 h. After evaporation of the solvents, the residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-1%) in dichloromethane] to give 0.55 g of 5'-O-acetyl-β-L-4-thiouridine (19a) sufficiently pure (tlc) to be used directly in the last step. The residue is dissolved in ammoniacal methanol (11 ml) and the solution is heated at 100°C for 3 h in an autoclave. The mixture is cooled, evaporated to dryness and the residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-12%) in dichloromethane] to give 0.33 g (yield 83%) of pure β-L-DDC (21a) which crystallizes from ethanol: m.p. = 220-222°C; UV (EtOH 95) max 273 nm, λmin 252 nm; ^{1}H-NMR (DMSO-d_6) δ ppm = 7.89 (d, 1H, H-6; J = 7.4 Hz), 7.15-6.95 (broad d, 2H, NH_2), 5.91 (dd, 1H, H-1'; J = 3.0 and 6.5 Hz), 5.66 (d, 1H, H-5; J = 7.4 Hz), 4.99 (t, 1H, OH-5'; J = 5.2 Hz), 4.05-3.95 (m, 1H, H-4'), 3.60-3.70 [m, 1H, H-5'; after exchange D_2O; dd, 3.64 ppm, J = 3.6 and 12.0 Hz], 3.60-3.50 [m, 1H, H-5"; after exchange D_2O: dd, 3.50 ppm, J = 4.1 and 12.0 Hz], 2.30-2.15 (m, 1H, H-2'), 1.9-1.65 (m, 3H, H-2", 3' and 3"); $[\alpha]_D^{20}$ -103.6 (c, 0.8, methanol); mass spectra (matrix: glycerol-thioglycerol, 50:50, v/v): FAB>0 423 $[2M+H]^+$, 304 $[M+glycerol+H]^+$, 212 $[M+H]^+$, 112 $[BH_2]^+$, 101 $[s]^+$; FAB<0 210 $[M-H]^-$.

Anal. Calculat d for $C_9H_{13}N_3O_3$ (M = 211.21): C 51.18; H 6.20; N 19.89. Found: C 51.34; H 6.25; N 20.12.

Example 6: 2',3'-Dideoxy-β-L-5-fluorocytidine (β-L-5-FDDC, 21b)

Acetic anhydride (0.34 ml; 3.60 mmol) is added at 0°C to a solution of β-L-5-FDDU (10b) (0.55 g; 2.39 mmol) in anhydrous pyridine (10 ml) and the reaction mixture is stirred for 1 h at 0°C and then for 3 h at room temperature. An additional quantity of acetic anhydride (0.22 ml; 2.33 mmol) is added and the stirring is continued for 3 h at room temperature. Ice-cold water is then added and the mixture is extracted twice with chloroform. The organic phase is dried over sodium sulfate, filtered, coevaporated several times with toluene, and evaporated to dryness to give 0.67 g of a residue corresponding to 5'-O-acetyl-β-L-5-FDDU (18b) sufficiently pure (tlc) to be used directly in the next step. Lawesson's reagent (0.60 g; 1.48 mmol) is added to a solution of the residue in anhydrous dichloroethane (67 ml) and the mixture is heated at reflux under argon. Two additional quantities of Lawesson's reagent are added, after 2 h (0.41 g; 1.01 mmol) and 3 h (0.20 g; 0.49 mmol) of reflux respectively. After evaporation of the solvents, the residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-2%) in dichloromethane] to give 0.48 g of 5'-O-acetyl-β-L-5-fluoro-4-thiouridine (19b) sufficiently pure (tlc) to be used directly in the last step. The residue is dissolved in ammoniacal methanol (12 ml) and the solution is heated at 100°C for 3 h 30 min in an autoclave. The mixture is cooled, evaporated to dryness and the residue is chromatographed on a silica gel column [eluent: step gradient of methanol (0-8%) in dichloromethane] to give 0.27 g (yield 51%) of pure β-L-5-FDDC (21b) which crystalliz s from ethyl acetate: m.p. = 158-160°C; UV (EtOH 95) λmax 281 nm (ε, 8400) and 237 nm (ε, 8500); min 260 nm (ε, 5700) and 225 nm (ε, 7800); ^{1}H NMR (DMSO-d_6) δ ppm 8.28 (d, 1H, H-6; J = 7.4 Hz), 7.7-7.4 (broad d, 2H, NH_2),

5.83 (poorly resolved dd, 1H, H-1'), 5.16 (t, 1H, OH-5'; J = 5.1 Hz), 4.05-3.95 (m, 1H, H-4'), 3.8-3.70 [m, 1H, H-5'; after exchange D_2O: dd, 3.71 ppm, J = 2.7 and 12.3 Hz], 3.60-3.50 [m, 1H, H-5"; after exchange D_2O: dd, 3.52 ppm; J = 3.3 and 12.3 Hz], 2.35-2.15 (m, 1H, H-2'), 1.95-1.75 (m, 3H, H-2", 3' and 3"); $[\alpha]_D^{20}$ -80.0 (-c 1.0, DMSO); mass spectra (matrix: 3-nitrobenzyl alcohol FAB>0 230 $[M+H]^+$; 130 $[BH_2]^+$ and 101 $[s]^+$; FAB<0 228 $[M-H]^-$.

Anal. Calculated for $C_9H_{12}N_3FO_3$ (M = 229.21): C 47.16; H 5.28; N 18.33; F 8.29. Found: C 46.90; H 5.28; N 18.07; F 8.17.

The compounds of the invention were subjected to pharmacological tests which show their benefit in the treatment of viral diseases.

Evaluation of the anti-HIV 1 activity on various cell lines.

HIV = human immunodeficiency virus.

The replication of HIV-1 (LAI isolate) in cell lines is measured by a reverse transcriptase (RTase) assay in the culture supernatant after 5 days of infection. This activity indicates the presence of a virus liberated by the cells. After adsorption of the virus, the test compounds are added at various concentrations to the culture medium.

The antiviral activity is expressed by the lowest concentration of compound which reduces the production of RTase by at least 50% (ED_{50}).

The toxic effect on the noninfected cells is assessed by colorimetric reaction based on the capacity of live cells to reduce 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide to formazan after incubating for 5 days in the presence of various concentrations of the compounds. The results are expressed as the lowest concentration of compound which causes at least 50% inhibition of the formation of formazan (CD_{50}).

β-L-DDC (21a) and even more so β-L-5FDDC (21b) have a marked ED_{50} on HIV-1 and HIV-2 as indicated below.

Composés :		<u>21a</u>	<u>21b</u>	AZT	DDC
CEM-SS/HIV-1 LAI	ED_{50}	3×10^{-7} M	3.8×10^{-8} M	2.5×10^{-9} M	3.5×10^{-8} M
	CD_{50}	8.3×10^{-5} M	9×10^{-5} M	$> 10^{-4}$ M	6×10^{-5} M
PBM/HIV 1 III B	ED_{50}	3.5×10^{-7} M	3×10^{-8} M	1.1×10^{-9} M	4×10^{-8} M
	CD_{50}	10^{-4} M	10^{-4} M	7×10^{-5} M	7×10^{-5} M
PBMC/HIV-2 D 194	ED_{50}	3.5×10^{-8} M	4.5×10^{-8} M	1×10^{-9} M	2×10^{-9} M
	CD_{50}	10^{-4} M	10^{-4} M	8×10^{-5} M	2.5×10^{-5} M

Furthermore, this anti HIV-1 activity is confirmed on various other cell lines:

MT-4 (ED_{50}: <u>21a</u> 1.5×10^{-5} M, <u>21b</u> 2.4×10^{-6} M; Ref: AZT 2.7×10^{-8} M, DDC 2.5×10^{-6} M)

U 937 (ED_{50}: <u>21a</u> 1.5×10^{-7} M, <u>21b</u> 4.2×10^{-10} M; Ref: AZT 4×10^{-10} M, DDC 3.8×10^{-10} M)

CEM TK^- (ED_{50}: <u>21a</u> 9.5×10^{-8} M, <u>21b</u> 9.5×10^{-8} M; Ref: AZT $> 10^{-4}$ M, DDC 2.5×10^{-6} M)

Finally, these compounds also exhibit an anti-HIV-1 activity on the lines resistant to AZT and Nevirapine

CEM-SS/HIV-1 Nevirapine resistant (ED_{50}: <u>21a</u> 10^{-6} M, <u>21b</u> 7.2×10^{-7} M; Ref: AZT 7.5×10^{-6} M, DDC 1.2×10^{-7} M)

MT2/HIV-1 AZT resistant (Larder) (ED_{50}: <u>21a</u> 3.5×10^{-7} M, <u>21b</u> 2×10^{-7} M; Ref: AZT 7×10^{-6} M, DDC 2.2×10^{-7} M)

<u>Legend to Figure 1</u>

Scheme I: Bases = purines or pyrimidines, optionally appropriately protected; R = Benzoyl (Bz), acetyl (Ac), monomethoxytrityl (mMTr), or tert-butyldiphenylsilyl